REMARKS/ARGUMENTS

Claims 120-138 are presented hereby in place of claims 101-119, cancelled hereby without prejudice or disclaimer.

Present claims 120-135, 137, and 138 correspond identically to claims 101-116, 118, and 119, respectively. Present claim 136 corresponds to claim 117 revised as suggested by the examiner in order to overcome §112 rejections of claim 117, as explained further below. Applicant wishes to thank the Examiner for helpfully suggesting alternative claim language for overcoming the rejections.

Claim 117 was rejected under 35 USC 112, ¶1, and under 35 USC 112, ¶2. Reconsideration is requested since claim 17 is amended hereby, as new claim 136, as suggested by the Examiner in order to overcome the rejection. That is, the language "in 20% ethanol, propanol, isopropanol, butanol, poly(ethylene glycol), or mixture thereof," in claim 117, is changed to "in a 20% alcoholic solution wherein the alcoholic solution is selected from the group consisting of ethanol, propanol, isopropanol, butanol, poly(ethylene glycol), and mixtures thereof," in claim 136. Accordingly, withdrawal of the rejections under §112, ¶1, and §112, ¶2, is in order.

Claims 101-119 were rejected under 35 USC 103 based on the combined teachings of Henco, Little, *International Dictionary of Medicine and Biology*, Vol. 1, 1986 (New York) page 522, and *Nucleic Acid Hybridisation - A Practical Approach*, 1985 (Washington D.C.) pages 64, 65, and 235 (Hames). Reconsideration is requested.

1. Examiner's recapitulation of salient argument.

Having regard to the situation faced now at the present state of the prosecution, it is highly necessary to compare in detail the presently claimed invention the cited references - in particularly, Henco (US 5,027,426) and Little (US 5,075,430). At the end, it is hoped, it will become clear that the question whether or not Henco's teaching (that the use of urea and detergent implicitly teaches the use of any substance that destroys the order of the water in which it is dissolved) would lead those skilled in the art to use this disclosure to arrive at the process of the presently claimed invention does not make any sense at all.

To avoid misunderstandings applicant, firstly, would like to refer to the teaching of the presently claimed invention and the claims which seem to be relevant with view to the above-mentioned clarification.

The presently claimed invention is directed to a process for the isolation and purification of nucleic acids from cells using two separation or purification stages as described in pending claim 120.

According to the first step a) the cells containing nucleic acids are digested and the cell debris is removed. Thereafter, the nucleic acids are treated with a low ionic strength buffer solution (first buffer solution) in the presence of an anionic exchanger whereby adsorption of the nucleic acids on the surface of the anionic exchanger is effected.

Thereafter, the nucleic acids are desorbed from the anion exchanger by applying a second buffer solution having a higher ionic strength than the first buffer solution. At the end this step b) the nucleic acids will be found in the eluate.

The second separation/purification stage is also divided into two partial steps - namely step c: and d:)

According to step c) the nucleic acids which have already been purified by applying the aforementioned step a) and b) (i.e., the nucleic acids are *inter alia* free from proteins etc.) the separated or purified nucleic acids are adsorbed onto the surface of a mineral support in the presence of the second buffer (eluate of step b)) optionally in the presence of lower alcohols.

In the last step d) the nucleic acids are desorbed again from the mineral support by applying an appropriate elution buffer - i.e. water or a third buffer solution having a lower ionic strength than the second buffer solution.

Regarding now claim 125. According to claim 125 a further washing step can be introduced between step c) and d) by applying an aqueous alcoholic solution.

According to claim 136 being dependent from claim 125 the alcoholic solution may include 1 to 7 M sodium perchlorate, 1 to 7 M guanidine hydrochloride, 1 to 6 sodium iodide or 1 M sodium iodide or 1 M sodium chloride in 20 % ethanol, propanol, iso-propanol, butanol, poly(ethylene glycol) or mixtures thereof.

It is no doubt true that the salts sodium perchlorate, guanidine hydrochloride, and sodium iodide belong to the group of chaotropic substances or chaotropes according to the so-called Hofmeister series. The counterpart of these chaotropes are the so-called kosmotropes.

The terms 'chaotrope' (disoder-maker) and 'kosmotrope' (order-maker) originally denoted solutes that stabilized, or destabilized respectively, proteins and membranes. Later they referred to the apparently correlating property of increasing, or decreasing respectively, the structuring of water.

Large singly charged ions, with low charge density (e.g. $H_2PO_4^-$, HSO_4^-, HCO_3^-, I^-, Cl^-, NO_3^-, NH_4^+, Cs^+, K^+ and tetramethylammonium ions; exhibiting weaker interactions with water than water with itself), are chaotropes whereas small or multiply-charged ions, with high charge density, are kosmotropes (e.g. SO_4^{2-}, HPO_4^{2-}, Mg^{2+}, Ca^{2-}, Li^-, Na^-, H^+, OH^- and HPO_4^{2-}, exhibiting stronger interactions with water molecules than water with itself).

From the above it clearly can be seen that sodium chloride cannot be classified as the "classic" chaotrope substance - on the contrary sodium chloride seems more to be qualified as a kosmotrope in the sense of the Hofmeister series.

Having in particular regard to this situation applicant takes liberty to refer to the fact that the examiner takes in the Office communication dated October 26, 2001 the point of view, that "sodium chloride clearly should be a chaotrope" [page 10, lines 19 to 25]:

> The Henco reference does not make specific reference to a chaotropic agent. In addition, the elution buffers used in Henco contain various proportions of NaCl, a compound notorious well-known in the art to alter the structure of water, and therefore NaCl must be also a chaotropic agent.

This is a fatal misinterpretation in the light of the Hofmeister series and it appears that the Examiner may be confused.

However, the question raised by the Examiner in the Office communication (dated September 17, 2002 now focuses on the use of urea as a chaotropic compound. The Examiner relies on two

additional references. From my point of view it cannot be seen that these two references would be of any help with regard to the proceedings or how these references support the Examiner's position.

The reason is as follows:

US '426 is concerned with a method for the separation of long-chain nucleic acids from other substances in solutions containing nucleic acids and other materials, comprising fixing long-chain nucleic acids in a nucleic acid-containing solution in a porous matrix, washing the porous matrix to separate the other substances from the long-chain nucleic acids, and removing the fixed long-chain nucleic acids from the porous matrix.

The object of Henco is [column 4, lines 38 to 61] to provide "a process for removing long-chain nucleic acids from tissues and body liquids which:

a) in a similar manner allows the nucleic acids to be extracted and concentrated from various starting materials, such as tissue, blood, sputum, cell cultures, bacteria, fungi, renal and fecal excrements, as well as from vegetable tissue from callus cultures, roots, etc.;

b) requires no long-time centrifugation steps, and more specifically no ultracentrifugation;

c) can be carried out without expensive equipment, and more specifically without refrigerated centrifuges and ultracentrifuges, and without using valuable material, such as caesium chloride for density gradients or rotor insertions for one-time use;

d) ensures high purity of the nucleic acid to be attained.

e) works without a phenolic extraction step; and

f) is suitable for being automated;

and by means of extraction of the long-chain nucleic acid, separates mixtures of long-chain nucleic acids and other materials, such as those obtained when products are biotechnologically produced."

Clearly, Henco refers to the use of urea at different places of the disclosure, mostly in cases where the biological samples for the long chain nucleic acid isolation are bacteria or viruses. For example:

Column 8, lines 59 to 63:

The method according to the invention utilizes the described porous matrix by lysing the CMV viruses in situ by addition of urea, detergent and buffer, whereupon the DNA (130 to 150.times.10.sup.6 Dalton) is released.

Column 12, lines 33 to 38 [Example 2]:

"Upon simultaneous addition of 4 M of urea, the DNA of the phages is released and, by means of another filtration through the cartridge, specifically adsorbed on the anion exchanger. Then the cartridge is washed with 0.8 M NaCl, 50 mM Tris-HCl buffe, pH 7.5, 1mM EDTA, and the DNA is eluted with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer, pH 7.5, 1 mM EDTA.

Column 12, lines 63 to 69 [Example 3]:

"...and the phage pellet is dissolved in 20 µl of 10 mM Tris, 1 mM EDTA, pH 7.5. Another part by volume of extraction buffer (2% Triton X-100®, 7 M urea, 100 mM EDTA, pH 7.5) is added, and the mixture is heated at 50 °C for 15 minutes to release the single-stranded DNA."

Column 13, lines 14 to 24 [Example 4]:

The isolation of cellular DNA from sperm is carried out as follows:

One hundred µl of sperm are suspended in 1 ml of 500 mM NaCl, 10 mM EDTA 40 mM DTE, 10 mM Tris-HCl buffer, pH 7.5, 1% Triton, 4 M urea and 20 µg/ml of proteinase K and incubated at 37 °C for 2 hours. After centrifugation at about 5000 g for 5 minutes, the supernatant is passed through the separating gel in a cartridge. The flow velocity of the supernatant through the cartridge is about 1 ml/min.

Column 13, lines 64 to 68 [Example 7]:

The preparation of CMV (cytomegalovirus) DNA from urine is carried out as follows:

CMV viruses are lysed in situ upon addition of 4 M urea, 1% Triton, 500 mM NaCl, 50 mM Tris-HCl buffer, pH 7.5

It must be stated that all these citations clearly show that Henco uses urea only for the purpose of lysis (as was already pointed out in our facsimile letter dated October 8, 1999). There is no hint to use urea as a chaoetropic substance in an elution buffer or for the purpose to wash nucleic acid adsorbed on a matrix. On the contrary, Henco discloses that the addition of urea to the loading buffer (i.e., before the adsorption/binding of the nucleic acid) has no effect the binding behavior of the long chain DNA.

This can clearly be seen from the description column 7, lines 8 to 11:

The addition of urea to the loading buffer does not affect the binding behavior of the long-chain DNA, while it optimizes the separation efficiency with respect to proteins.

As can be seen, the only advantageous effect of the addition of urea results in a better separation efficiency with regard to the proteins. However, this is of no help at all with regard to the solution of the problem underlying the presently claimed invention. Even if one would like to argue that the addition urea to the loading buffer has positive effects with regard to the separation of nucleic acid from proteins during the following elution from the porous matrix and if - assuming arguendo - one would like to follow the line of argumentation of the Examiner that urea implicitly mans the use of any substance which destroys the order of water in which it is dissolved (i.e., a chaotrope) the advantageous effect of protein separation will only show relevant for unpurified lysates.

It must be noted that according to the presently claimed invention the mixture resulting from step b) and being the starting material of step c) is already purified and, thus, is free from proteins. Accordingly it cannot be seen that the teaching of Henco is of any help for the solution of the problem of the presently claimed invention because the use of urea or a chaotrope at such a late step is simply superfluous in the light of the disclosure of the U.S. patent '430.

However, it cannot be seen that the use of urea as a lysis agent leads those skilled in the art to use chaotropic substances in a washing buffer, to wash nucleic acids which are bound on a matrix.

Keeping this in mind the examiner seems to fight on another battlefield. It should be born in mind that a skilled reader will study a document in a practical manner and in an afford to make sense of it. Thus, the skilled reader will discard any possible interpretations of the document which are illogical, impractical or fanciful. If the Henco citations is read in this manner, it will immediately be evident that Henco is no good point to start from if a skilled man is going to solve the problem underlying the presently claimed invention.

Looking now at U.S. Patent 5,075,430 (Little)

This patent is concerned with a process for the purification of plasmid and other DNA, both single-stranded and double-stranded, by immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent and eluting the DNA with water or low salt buffer and a process for the immobilization of DNA onto diatomaceous earth in the presence of a chaotropic agent.

The invention is based on the finding that that diatomaceoius earth is useful for the purification of plasmid and other DNA by immobilizing the DNA onto the diatomaceous earth

particles in the presence of a chaotropic agent, following by elution of the DNA with water or low salt buffer.

More particularly, the Little citation discloses a process for the purification of plasmid DNA comprising the following steps:

a) *immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent;*

b) *washing the resulting diatomaceous earth-bound DNA with an alcohol-containing buffer;*

c) *removing the alcohol-containing buffer; and*

d) *eluting the DNA in a low salt buffer or in water.*

As already mentioned above, the invention is also directed generally to the immobilization of DNA onto diatomaceous earth which comprises contacting the DNA with the diatomaceous earth in the presence of a chaotropic agent.

According to the invention a chaotropic substance is to be understood as a

> *"... substance that enhances the partitioning of nonpolar molecules from a nonaqueous to an aqueous phase as a result of the disruptive effect that the substance has on the structure of water. Examples of chaotropic agents include sodium iodide, sodium perchlorate and sodium trichloroacetate."*

[column 3, lines 36 to 43] (It should be noted again that sodium chloride forms not a part of the above mentioned chaotropic sodium salts).

It is no doubt true that the chaotropic substance is primarily used to bind the nucleic acid on the diatomaceous earth matrix.

However, Little discloses also to use a chaotropic binding buffer and a 50% ethanol buffer [column 4, lines 21 to 35]:

> *An example of an alcohol-containing wash buffer comprises: 20.0 mM Tris-Cl pH 7.5, 20 mM EDTA, 0.4 M NaCl, and 50% v/v ethanol. This buffer will be abbreviated herein as "50% ethanol buffer" or "50% washing buffer".*
>
> *In order to lower the RNA and protein concentration in plasmid lysates, it is necessary to perform a sufficient number of washes using the chaotrope binding buffer and the 50% washing buffer. The amount of RNA and protein remaining is indirectly proportional to the number of volume washes performed on the diatomaceous earth pellet, membrane or column. Generally, about three washes of each buffer is sufficient to lower the RNA and protein concentrations to acceptable levels.*

From the above it clearly can be seen that the washing steps using the chaotropic buffer on one hand and the washing buffer on the other hand are necessary to lower hte RNA and protein concentrations in plasmic lysates. However, similar to Henco, this step is necessary when using complex and not pre-purified lysates as starting material.

On the other hand it must, again, be noted that according to the presently claimed invention the mixture resulting from step b) and being the starting material for step c) is purified and, thus, is free from RNA and proteins.

Moreover, it should be noted that Little's intention with regard to the elution of the DNA was to optimize the yield of DNA to be recovered [column 4, lines 36 to 46]:

> *The efficiency of release of immobilized DNA from the diatomaceous earth pellet, membrane or column will be proportional to the ratio of the volume of low-salt buffer or water added to the volume of the pellet, membrane or column. Thus, with a 5 µl diatomaceous earth pellet, for example, 5 µl of buffer or water (1 volume) will extract about 50% of the DNA. Likewise, 10 volumes of buffer or water added per volume of pellet will permit the recovery of >90% of the DNA. However, it should be kept in mind that the more buffer or water added, the more dilute the eluted DNA.*

Accordingly, Little does not face at all the problem to isolate the DNA in a solution having a low salt concentrate.

Thus, in my submission, the skilled artisan would not find Little a good place to start from when looking for teaching to solve the problem of the presently claimed invention. Furthermore, there was absolutely no motivation for a man skilled in the art to combine the teaching of both references - which both are concerned with DNA isolation from crude starting material - to reach the two step process of the presently claimed invention.

Even if - what is denied - one skilled in the art would have taken into account such a combination, it is clear - as discussed above - that he has to be inventive to find all the new parameters for such a two step isolation. The basic knowledge that urea does belong to the group of chaotropic substances would be of no or no essential help to arrive at a solution of the problem underlying the presently claimed invention.

2. Examiner's new rebuttal arguments

The Examiner refers on page 13, lines 14 to 22 argues:

> *Therefore, Henco '426 use a combination of "urea, detergent and buffer" to effect lysis of CMV viruses (column 8, lines 60-62) is plainly an example wher etow of the three components of the lysis buffer are chaotripic (urea and detergent), a reality which hte lsysi buffer relies on the effect on cell wall lysis. Examiner concludes that applicant's argument is not convincing because there is no requirement that the prior art use any particular term to specify in the absence of the presence of specific term.*

In my submission it should be noted that this statement quite disturbing, to say the least. As was shown above, urea, when was use din henco '426, it was used in all cases in the lysis step. According to this disclosure it is no doubt true that the teaching of Henco clearly is, to use urea for the lysis (and

not for the elution). The side effect of the better separation of the proteins from the nucleic acid is - as was shown of no relevance for the use of some special chaotropic substances (as listed in claim 116) according to the presently claimed invention.

What the Examiner tries is to take the word "urea" completely out of context of the teaching of Henco '426, to search for a specific inherent feature (which is of no meaning for the use disclosed by Henco) and in the last step - after generalization (chaotropic substances) - to find a_?_ - according to his opinion - appropriate other feature and use of such class of compounds (as washing buffers) and to build up a very artificial and highly impermissible hindsight reconstruction only based on this new feature.

Favorable action is requested.

Respectfully submitted,

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